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Short Communication

Partial Sequence Analysis of the 5S to 18S rRNA Gene Region of the Maize Mitochondrial Genome[1]

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ABSTRACT

The nucleotide sequence has been determined for a 664-base pair region of maize (*Zea mays* L.) mtDNA which contains the 3′ end of the 18S rRNA gene, the 5S rRNA gene, and an intergenic region of 108 base pairs. Specific regions of the 18S rRNA gene show striking homology with the corresponding gene in *Escherichia coli*.

The organization and structure of the rRNA genes in plant mitochondria are different from those of animals and fungi. In plant mitochondria, rRNA genes are larger and a 5S RNA is found in mitochondrial ribosomes (6, 15). The 5S and 18S rRNA genes in plant mtDNA are closely linked (3, 10, 12), but both are separate from the 26S rRNA gene (24). In maize, the 26S rRNA gene is separated from the 18S and 5S by 16,000 base pairs (bp) of DNA (24). Each of the rRNA genes is encoded by a single gene (24).

This organization of 18S and 5S rRNA genes is unique to plant mtDNA. Prokaryotes have clusters of 16S, 23S, and 5S genes in that order. Chloroplasts usually have an organization like prokaryotes. Most eukaryotic nuclei have clusters of 5S genes which are unlinked to the 18S and 28S rRNA genes (17). To study the structure of plant mitochondrial rRNA genes, we have isolated a cloned fragment which contains the linked genes of 18S and 5S rRNA and determined a portion of the nucleotide sequence.

The section of the molecule which has been sequenced contains part of the 18S rRNA gene and a complete 5S rRNA gene. We have located the 5S gene by sequence homology using the published sequence of the 5S rRNA from the mitochondria of wheat (22). Similarly, the location of the 3′ end of the 18S gene was found because of strong homology with the corresponding genes of *E. coli* and maize chloroplast. The location of these boundary sequences defines the intergenic region which is 8 bp long and establishes the orientation and direction of transcription of both 18S and 5S rRNA genes.

The large degree of homology found between the mitochondrial sequence and the bacterial sequence provides further support for the view that plant mitochondria have an endosymbiotic-eubacterial origin (2, 9, 10).

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MATERIALS AND METHODS

Cloning of Maize mtDNA. Normal mtDNA, purified from maize (*Zea mays* L.) from single cross NC7 × T204, was digested with restriction enzyme Bam HI and cloned into *Escherichia coli* plasmid pBR322. Detailed methods for these techniques including restriction digestions and Southern transfers have previously been described (23).

Purification and Labeling of rRNA. Mitochondria were isolated from coleoptile and mesocotyl tissues according to the procedures of Cunningham and Gray (7). Ribosomes were pelleted through sucrose and deproteinized by phenol extraction. RNA subunits were fractionated by electrophoresis in methylmercury gels (1) and recovered from agarose according to Locker (16). Purified RNA was labeled with polynucleotide kinase following partial alkaline hydrolysis (0.1 N KOH, 4°C for 1 h).

Cloning and Sequencing with M13. Recombinant clones were isolated using phage M13 (18). Small fragments from subclone 542-2, digested with Taq I, Sau 3A, and Hae III were cloned into vectors M13mp7, mp8, and mp9 developed by Messing (18). Sequencing reactions used a universal primer and the dideoxy chain termination procedures of Sanger *et al.* (19). Electrophoresis was performed on 8% polyacrylamide gels (0.4 mm) at 1,600 to 2,000 v for 3 to 6 h at a gel temperature of approximately 50°C. Autoradiography was done at −20°C using Kodak X-Omat AR film. Computer analysis of the intergenic region was done by using the SEQ program of tbe Stanford MOLGEN project (5).

Calculation of Homology. The maize mtDNA sequence has been aligned with sequences of the 16S rRNA genes of maize

Table I. *Calculated Homologies for 3′ Sequence of Several Small Subunit rRNAs*

	Homology		No. of Nucleotides
		%	
Maize mt to *E. coli*	Gap included	65.3	300
	Gap excluded	77.5	253
Maize ct to *E. coli*	Gap included	74.8	254
	Gap excluded	74.8	254
Maize mt to Maize ct	Gap included	62.0	300
	Gap excluded	73.2	254
Human mt to *E. coli*	Gap included	45.7	254
	Gap excluded	52.7	220
Yeast mt to *E. coli*	Gap included	47.5	278
	Gap excluded	52.2	253

chloroplast (21) and *E. coli* (4). Gaps were introduced in the −160 to −80 region, to align the sequence. Homology has been calculated in two ways. The 'gap included' homology is calculated considering all gaps as nonmatching base pairs. For the 'gap excluded' homology, gaps of seven or more bp are treated as deletions and are excluded from the calculations. Alignments of the yeast, chloroplast, and human sequences with *E. coli* (not shown) were taken from Kuntzel and Kochel (14) for Table I.

RESULTS

The ZmmtN542 Cloned Fragment Contains the 18S rRNA Gene. Labeled rRNA genes from maize mitochondria were used to identify a single Bam HI fragment cloned from normal mtDNA that hybridized to 18S rRNA but not to 26S rRNA. Nick translation of the clone and hybridization showed homology to one band in a Bam HI digest of normal mtDNA (23). The fragment, designated ZmmtN542, is estimated to be 16,000 bp. Huh and Gray (12) have hybridized labeled 18S rRNA from wheat (*Triticum aestivum* L.) to a Bam HI digest of maize mtDNA and found a single labeled band in the same position as clone 542. Labeled wheat 5S rRNA hybridized to the same large fragment suggesting physical linkage, while 26S rRNA hybridized to a different band. The 18S and 5S genes of maize remained linked after digestion with five different hexanucleotide restriction enzymes (12) suggesting that the genes are linked by less than 1,000 bp, assuming one restriction site in 4,096 bp for each hexanucleotide restriction enzyme.

Clone 542 Contains One Copy of the 18S Gene. Digestion of clone 542 with restriction enzymes followed by hybridization with labeled 18S rRNA in Southern transfer experiments shows that the 18S gene is located in a 2,000 bp fragment (Fig. 1) bounded by Pst I and Hind III sites. Only one copy of an 18S rRNA gene could be located within this fragment.

For more detailed analysis, clone 542 was digested with Pst I and the 3,200 bp fragment was subcloned into the Pst I site of pBR 322. The subclone was designated 542-2. The restriction map of the subclone is very similar to the map of the 18S gene in wheat (10).

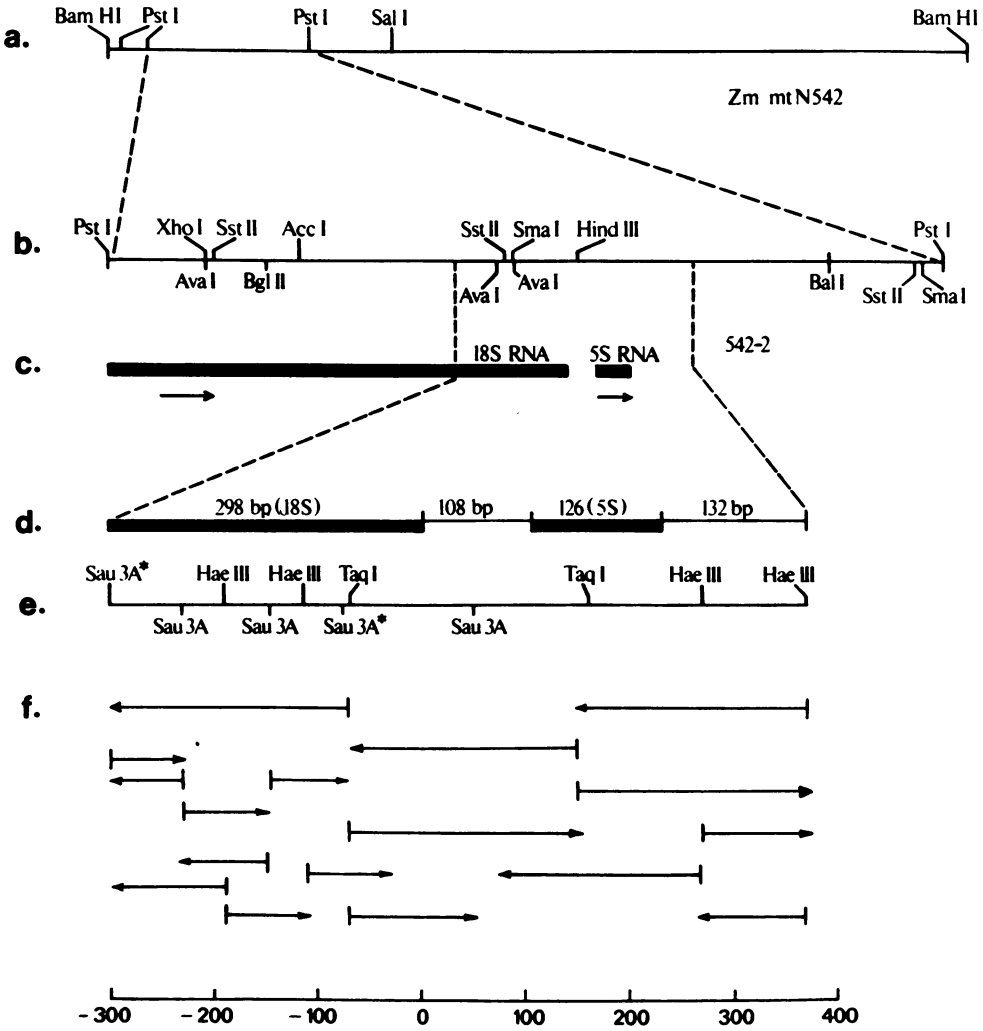

FIG. 1. Restriction mapping and sequencing strategy. (a), Restriction map of Bam HI fragment ZmmtN542. Length of cloned fragment is 16,000 bp. (b), Expanded map of the 3,200 bp Pst I fragment (subclone 542-2) that contains the 18S rRNA gene. (c), Regions of Pst I fragment (542-2) in (b) which code for 18S rRNA and 5S rRNA. Scale is the same as (b). Arrows indicate the direction of transcription (5′ to 3′). (d), Expanded regions transcribed into RNA and flanking sequences are shown with the lengths of the regions sequenced. (e), Fine structure restriction map of the sequenced segment of 542-2. (f), Expanded region of 664 bp within the Pst I subclone (542-2). Sequencing strategy shows the direction of sequencing from fixed restriction sites. Each arrow represents a cloned fragment from this region which was sequenced in that direction. Arrows going in opposing directions indicate sequence of complementary strands. Sau 3A* denotes a site cut by Sau 3A which is either GGTC or GATT instead of the GATC sequence. Sau 3A preparations have a secondary activity on these sequences, perhaps due to high glycerol concentration during digestion.

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                0          10          20          30          40          50
               -300                                                      -250
Maize mt.      TTG-CCTCAG  TTCGGATTGT  TCTCTGCAAC  TCGGGAACAT  GAAGTAGAAA
Maize ct       CCGTCCTCAG  T-CGGATTGC  AGGCTGCAAC  TCGCCTGCAT  GAAGCAGGAA
E. coli        GCGTCGT-AG  TCCGGATTGG  AGTCTGCAAC  TCGACTCCAT  GAAGTCGGAA

               -250                                                      -201
Maize mt.      TCGCTAGTAA  TCGCGGATCA  GC-ATGCCGC  GGTGAATATG  TACCCGGGCC
Maize ct       TCGCTAGTAA  TCGCCGGTCA  GCCATACGGC  GGCGAATCCG  TTCCCGGGCC
E. coli        TCGCTAGTAA  TCGTGGATCA  G-AATGCCAC  GGTGAATACG  TTCCCGGGCC

               -200                                                      -151
Maize mt.      CTGTACACAC  CGCCCGTCAC  ACCCTGGGAA  TTGGTTTCGC  CCGAAGCATC
Maize ct       TTGTACACAC  CGCCCGTCAC  ACTATAGGAG  CTGGCCAGGT  TTGAAG----
E. coli        TTGTACACAC  CGCCCGTCAC  ACCATGGGAG  TGGGTTGCAA  AAGAAG----

               -150                                                      -101
Maize mt.      GGACCAATGA  TCACCCATGA. CTTCTGTGTA  CCACTAGTGC  CACAAAGGCC
Maize ct       -------TCA  GTAGCCTTAA  ----------  ----------  --------CC
E. coli        -------TAG  TTACC-TTAA  ----------  ----------  --------CC

               -100                                                       -51
Maize mt.      TTTGGTGGTC  TTATTGGCGC  ATACCACGGT  GGGGTCTTCG  ACTGGGGTGA
Maize ct       GTAAGGAG--  -----GGGGA  TGCCTAAGGC  TAGGCTTGCG  ACTGGAGTGA
E. coli        TTCGGGAG--  -----GGCGC  TTACCACTTT  GTGATTCATG  ACTGGGGTGA

               -50                                                        -1
Maize mt.      AGTCGTAACA  AGGTAGCCGT  AGGGGAACCT  GTGGCTGGAT  TGAATCCTTC
Maize ct       AGTCGTAACA  AGGTAGCCGT  ACTGGAAGGT  GCGGCTGGAT  CACCTCCTTT
E. coli        AGTCGTAACA  AGGTAACCGT  AGGGGAACCT  GCGGTTGGAT  CACCTCCTTA

               1                                                          50
Maize mt.      GCGATGGTCG  CCTACTTGAC  TAAACTAAGG  ACCTCAACGG  AAGCTTATGT

               51                                                        100
Maize mt.      AGATCGACTG  AAAAGACGAT  TCCATTTTCC  TTCTTGTTTA  TCAATCACCA

               101                                                       150
Maize mt.      ATCAAGACAA  ACCGGGCACT  ACGGTGAGAC  GTGTTTACAC  CCGATCCCAT.
Wheat mt 5S    --------AA  ACCGGGCACU  ACGGUGAGAC  GUGAAAACAC  CCGAUCCCAU

               151                                                       200
Maize mt.      TCCGACCTCG  ATATATATGT  GGAATCGTCT  TGCGCCATAT  GTACTGAAAT
Wheat mt 5S    UCCGACCUCG  AUAUAUAUGU  GGAAUCGUCU  UGCGCCAUAU  GUACUGAAAU

               201                                                       250
Maize mt       TGTTCGGGAG  ACATGGTCAA  AGCCCGCCCC  TAAAGGGGAT  TTCTTTCAGC
Wheat mt 5S    UGUUCGGGAG  ACAUGGUCAA  AGCCCGG---  -AAA------  ----------

               251                                                       300
Maize mt       CAACATCCTG  CCTGCCTTAG  TGGCCCCTCT  CTGATAAGGA  AACGAAAAAA
Wheat mt 5S    ----------  ----------  ----------  ----------  ----------

               301                                                       350
Maize mt.      AAATGACAAA  TCTGGTCCTT  TCAACTGTCT  GTACTAAATT  ACTTTGTACG

               351         366
Maize mt.      AATGCACATC  TCGGCC
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FIG. 2. Nucleotide sequence of maize mtDNA containing the 3′ end of 18S rRNA gene, the 5S rRNA gene, and flanking sequences is shown. The regions at the end of 18S rRNA gene are numbered from zero. The beginning and end of the 5S rRNA gene are at number 109 and 234. The sequence of the maize ct (chloroplast) 16S rRNA comes from Schwartz and Kossel (21) and the *E. coli* sequence is that of Brosius *et al.* (4). Alignments of the sequences follow that of Kuntzel and Kochel (14) for chloroplast and *E. coli*. The underlined region at number 46 is a possible promotor sequence. The DNA strand equivalent to the RNA sequence is shown.

Nucleotide Sequence of the 18S to 5S Region. M13 clones, containing fragments of subclone 542-2 cut with Taq I, Hae III, or Sau 3A were sequenced by the Sanger procedure (19). The fragments were oriented based on overlapping sequences and by the location of known restriction sites (Fig. 1). To determine the direction of transcription and the orientation of the sequence on the map, the M13 clones were hybridized with labeled 18S rRNA. Those clones which hybridized to the RNA contained the transcribed strand, and their complements were equivalent to the 18S rRNA. The nucleotide sequence (Fig. 2) was determined from both strands.

Location of the 3′ End of the 18S Gene. Previous work has shown extensive homology and conservation of sequence for the small subunit rRNA (14), including cross hybridization of rRNA of *E. coli* with maize mtDNA (13). We therefore searched our sequences for homology with the ends of *E. coli* 16S rRNA and located a region at one end of the 18S gene of 298 bp near the Hind III site of the 542-2 subclone, which is homologous to the 3′ end of the *E. coli* 16S rRNA gene. Gray *et al.* (20) have determined the terminal 100 nucleotides of the 18S rRNA of wheat; their sequence is identical to the maize DNA sequence.

Location of the 5S Gene. Since Huh and Gray (12) had shown close proximity of the 5S gene with the 18S sequence, and the 5S sequence of wheat mtDNA was published (22), we searched the flanking sequences of the 542-2 clone distal to the Hind III site for the 5S sequence. The homologous sequence was found at positions 9 to 234. Both 18S and 5S genes are transcribed from the same strand.

Properties of the Intergenic Region. The intergenic region between the 5S and the 18S rRNA genes contains 108 bp. A Pribnow-like sequence TATGTAG is located at nucleotide 46 near the Hind III site. A short open reading frame of 35 codons is found on the RNA equivalent strand using either the universal code or a mitochondrial code (not shown), beginning with a methionine initiation codon four bases after the 3′ end of the 18S gene. Computer searches for symmetry and complementarity have revealed some internal structure when GU pairing is assumed. A tRNA gene sequence was not observed, but the possibility of a somewhat unusual tRNA is not precluded.

DISCUSSION

Our data confirm and extend previous results which indicate that the organization of the mitochondrial rRNA genes is quite different from the rRNA genes of the chloroplast and nucleus in maize. In spite of the close eubacterial homology observed in the 18S sequence, the organization of rRNA genes is also different from the prokaryotic prototype.

Earlier, Gray and Doolittle (11) argued that the strongest evidence for the proposed prokaryotic nature of mtDNA was derived from T_1 oligonucleotide comparisons of wheat mitochondrial 18S rRNA with eubacterial 16S and other small subunit rRNAs. More recently, Schnare and Gray (20) have sequenced the last 100 nucleotides of the 18S rRNA of wheat mitochondria and have demonstrated directly the high degree of homology. We now present the maize 18S sequence for 298 nucleotides from the 3′ end, and show that homology varies greatly in different regions of the molecule. For example, from −300 to −180, the homology with *E. coli* is 81.7%. From −180 to −60, the homology is decreased to 62.2% excluding gaps and to 38.3% if gaps are included. The last 60 nucleotides have 86.7% homology. Variation in nucleotide conservation among different regions of the small subunit rRNA molecule has been observed in other systems (14). Measurements of rates of divergence using mathematical models which presume uniformity are not satisfactory in these circumstances. In addition, we compare sequences of the small rRNA subunit from both mitochondria and chloroplast of maize. Both the chloroplast and the mitochondrial sequences contain extensive homology with *E. coli*. (Table I). In contrast, the rRNA genes of animal or fungal mitochondria show much less similarity with *E. coli*. (Table I). These distinctions suggest that divergence has been more rapid in other mitochondria or that mitochondria have had a complex evolutionary history.

The 3′ end of the maize mitochondrial 18S rRNA gene does not contain the Shine-Dalgarno sequence found at the 3′ end of *E. coli* 16S rRNA (CCUCC). However, another sequence TGAAT, located close to the end, may be an equivalent sequence. The complementary sequence ATTCA is found at the 5′ end of the maize mitochondrial gene for Cyt oxidase subunit II, at −22 to −18 bp from a possible methionine initiation codon (8). Sequence analysis of other mitochondrial genes is needed to determine if the ATTCA sequence truly represents a ribosome binding site for plant mitochondrial mRNA genes.

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